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David E. Mann, Jr., Ph.D.
Temple University

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Pathology Report

Effects of Tobacco Smoke and Tobacco Residue on Methylcholanthrene-Induced Skin Carcinogenesis in Mice

By: Dr. Frederic James, Temple University School of Dentistry, Philadelphia, Pa.

In compiling a pathological report on this research project suffice it to say that a preliminary examination was undertaken microscopically on all tumor sections regardless of the group in which the mouse was classified. This was done to determine variations, if any, as to the type of tumor. As it happened all growths, regardless of their size or location showed very little departure from a generalized pattern. The same held true during the period the animals were living. In this instance the only variations seen were those of shape, size and consistency as is well demonstrated by the selected series of Kodachrome prints. It was very interesting to note as growth continued that some assumed a raised circular characteristic, while others showed variable horn-like formations. In most instances the growths were loosely attached to the deeper structures and when removed for fixation often fell apart due to central caseation, although on the surface most of them were hard as evidence of hyperkeratosis. Once a growth was removed from its attachment to the thoracic wall, which was the case in most instances, the thoracic organs were free within their cavity. This seemed quite remarkable in view of the extensive nature of so many of the growths. In many cases the head of the animal was either drawn to the right or left side or held positionally central when the growth almost ringed the neck. Invariably the ear and forelimb or ears and forelimbs were contained in the mass, but regardless of all these variations in a forward, lateral or backward direction the growths appeared to be quite superficial. It was remarkable to note that only in a few instances at the time of autopsy was there any evidence of secondary (metastatic) growths in lung, liver, kidney or pancreas. Even in cases where nodules were suspected the matter was only given tentative consideration pending microscopic examination.

Each section examined microscopically showed the following characteristics to a greater or lesser degree, depending upon the size of the neoplasm and the time of death, whether occasioned by the growth or induced at the end of the experimental period:-

1. A progressive increase in the thickness of the stratum corneum which in practically every instance presented an extreme hyperkeratosis.

2. Broadening, with resultant fusion of the interpapillary processes in addition to an extreme depth of penetration into the submucosa.

3. The cells of the invading epithelium varied considerably in-so-far-as:-

   (a) The outermost layer resembled the basal layer of the epithelium

(b) The innermost layers lacked, in most instances, a clear differentiation. Some of the cells were round, others elongated with variations in their cytoplasmic staining.

(c) Many of the nuclei of the cells exhibit hyperchromatism.

(d) Mitoses were numerous and for the most part atypical.

4. "Cell nests" or squames are abundant and in the superficial layers of the neoplasm are represented as keratinized cores devoid of any cellular elements.

5. Squames in the deeper regions show more definition since they are composed of several layers of flattened "prickle-cells" concentrically arranged about a core of homogenous acidophile hyaline.

6. Many of the "cell nests" by virtue of their breakdown consist of fragments of keratinous material, as well as granular and necrotic debris.

7. Numerous polymorphonuclear leucocytes infiltrate into some of the more recently formed "cell nests" although the presence of these cells is not limited since they are seen in greater numbers scattered among the epithelial cells and the connective tissue elements.

8. Between the epithelial processes the connective tissue varies. In some areas it is loose in texture, in others it is definitely fibrous in character.

9. In the tissue spaces many epithelial cells have become detached, either singly or in clusters and are intermingled with the inflammatory cells which are composed for the most part of polymorphonuclear leucocytes along with lymphocytes, plasma cells and histiocytes.

10. Deposits of pigment, hemosiderin, are quite abundant.

11. Necrotic tissue that presented, prior to sectioning, a soft dryish, crumbly, cheesy mass due mainly to fatty degenerative changes. This sphacelus in some areas is devoid of any structural characteristics, in others it is admixed with degenerated cellular elements of both tissue and blood (leucocytes).

12. The predominance of polymorphonuclear leucocytes presented a most interesting microscopic picture in most instances.

In summarizing this series of microscopic examinations one can only repeat that in spite of the grouping of the animals and the methods employed in applying the methylcholanthrene the neoplasias per se followed a pattern of similarity in that structural form and appearances while the animals lived did not produce any difficulties in diagnosis in that squamous-cell carcinoma was the rule. It can also be confirmed that in those cases that manifested true metastases in the lungs, the diagnosis was the same.

ANALYSIS OF SECTIONS OF LUNG

GROUP "A"

No. 1. Apparently little change from the normal pattern.

No. 2. Interstitial pneumonitis with some bronchiolitis.

No. 3. Interstitial exudate and interstitial cellular infiltration. Hyalinization with eosinophilic staining. Degenerated polymorphonuclear leucocytes.

No. 4. Primarily a pneumonitis.

No. 5. Interstitial broncho-pneumonia.

No. 6. Broncho-pneumonia. Alveolar obliteration. Extreme cellular infiltration. Consolidation in many areas.

No. 7. Apparently little change from the normal pattern.

No. 8. Acute pneumonitis and bronchiolitis.

No. 9. Acute pneumonitis and bronchiolitis.

No. 10. Apparently little change from the normal pattern.

No. 11. Apparently little change from the normal pattern.

No. 12. Acute pneumonitis and bronchiolitis. Hyalinization with some consolidation.

No. 13. Slight pneumonitis.

No. 14. Acute pneumonitis with marked cellular infiltration.

No. 15. Slight pneumonitis.

No. 16. EXTREME METASTASIS COMPARABLE TO PRIMARY TUMOR ON THE SKIN. GREATER PART OF LUNG INVOLVED. KERATINIZED "CELL NESTS" ABUNDANT AND PATTERN TYPICAL.

No. 17. Apparently little change from the normal pattern.

No. 18. Acute pneumonitis and bronchiolitis.

No. 19. Slight pneumonitis and bronchiolitis.

No. 20. Slight pneumonitis.

No. 21. Pneumonitis.

No. 22. Apparently little change from the normal pattern.

No. 23. MARKED METASTASIS IN THE LUNG TISSUE AND ABOUT THE BRONCHI AND BRONCHIOLES. DEGENERATION IN CENTRAL PORTION OF TUMOR. "KERATINIZED CELL NESTS" PRESENT BUT SMALL TO MEDIUM IN SIZE.

No. 24. Acute pneumonitis and bronchiolitis. Much evidence of hemosiderin.

No. 25. METASTASIS COMPARABLE TO PRIMARY TUMOR ON THE SKIN. KERATINIZED "CELL NESTS" WELL DEVELOPED AS WELL AS OTHER MALIGNANT CHARACTERISTICS OF THE CELLULAR ELEMENTS - ANASPLASIA, PLEOMORPHISM AND HYPERCHROMATISM.

No. 26. Broncho-pneumonia. Excessive exudate. Cellular infiltration. Areas of emphysema.

No. 27. Acute pneumonitis and bronchiolitis.

No. 28. Broncho-pneumonia with marked exudate and cellular infiltration.

No. 29. Apparently little change from the normal pattern.

No. 30. Pneumonitis and bronchiolitis.

NOTE:

THE ABOVE TABULATION REPRESENTS THE GENERAL ANALYSIS OF GROUP "A" SERIES AND REVEALS UPON MICROSCOPIC EXAMINATION THAT:

No. 16

No. 23 ALONE IN THE SERIES SHOWED METASTATIC DEPOSITS IN THE LUNG.

No. 25

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ANALYSIS OF SECTIONS OF LUNG

GROUP "B"

No. 31. Apparently little change from the normal pattern.

No. 32. Pneumonitis and cavitation of lung tissue. Interstitial cellular infiltration.

No. 33. Acute pneumonitis and extreme bronchiolitis. Congestion very marked.

No. 34. Apparently little change from the normal pattern.

No. 35. EXTREME METASTASES COMPARABLE TO PRIMARY TUMOR ON SKIN. KERATINIZED "CELL NESTS" WELL DEFINED IN ADDITION TO OTHER MALIGNANT CHARACTERISTICS.

No. 36. Pneumonitis with apparently some cavitation of lung tissue.

No. 37. Apparently little change from the normal pattern.

No. 38. Apparently little change from the normal pattern.

No. 39. EXTREME METASTASIS ALMOST OBLITERATING LUNG TISSUE. LARGE NUMBER OF KERATINIZED "CELL NESTS" SHOWING CENTRAL NECROSIS.

No. 40. Pneumonitis.

No. 41. Pneumonitis and bronchiolitis. Abundant deposits of hemosiderin.

No. 42. Acute broncho-pneumonia. Exudate excessive. Marked interstitial cellular infiltration. Alveoli distended and many show tissue breakdown.

No. 43. Pneumonitis.

No. 44. A SECTION OF THE LUNG TISSUE SHOWS A SMALL AREA OF METASTASIS. KERATINIZED "CELL NESTS" ARE PRESENT BUT ARE VERY IMMATURE.

No. 45. Pneumonitis and bronchiolitis.

No. 46. SMALL AREA OF METASTASIS EXTENDING INTO LUNG TISSUE FROM THE SURFACE OF LOBE. COMPOSED MAINLY OF MALIGNANT CELLS. NO KERATINIZED "CELL NESTS."

No. 47. Apparently little change from the normal pattern.

No. 48. DEFINITE METASTASIS COMPARABLE TO PRIMARY TUMOR ON SKIN. KERATINIZED "CELL NESTS" AND OTHER MALIGNANT CHARACTERISTICS.

No. 49. Acute pneumonitis and bronchiolitis.

No. 50. Apparently little change from the normal pattern.

No. 51. Small patches of evident pneumonitis with some emphysematous changes.

No. 52. EXTREME METASTASES COMPARABLE TO PRIMARY TUMOR ON SKIN. KERATINIZED "CELL NESTS" IN ADDITION TO BEING LARGE ARE ALSO NUMEROUS AND REVEAL VARIOUS PATTERNS. TWO METASTATIC AREAS ARE DEMONSTRABLE. MALIGNANT CELLS ARE ANASPLASTIC, PLEOMORPHIC AND SHOW MARKED HYPERCHROMATISM.

No. 53. Apparently little change from the normal pattern.

No. 54. Pneumonitis and bronchiolitis.

No. 55. Slight pneumonitis. Lung tissue shows little involvement.

No. 56. Pneumonitis. Lung tissue shows considerable vascular congestion.

No. 57. MARKED METASTASIS WITH VARIABLE SIZE KERATINIZED "CELL NESTS" AND OTHER MALIGNANT CHARACTERISTICS. THE TUMOR IS COMPARABLE IN GENERAL DESIGN TO THE PRIMARY TUMOR ON THE SKIN.

No. 58. Slight pneumonitis. Changes not too well defined.

No. 59. Pneumonitis and bronchiolitis. Extensive cellular infiltration.

No. 60. Pneumonitis and bronchiolitis with extreme edema and congestion.

NOTE:

THE ABOVE TABULATION REPRESENTS THE GENERAL ANALYSIS OF GROUP "B" SERIES AND REVEALS UPON MICROSCOPIC EXAMINATION THAT:

No. 35
No. 39
No. 44
No. 46 ALONE IN THE SERIES SHOWED METASTASES IN THE LUNG
No. 48
No. 52
No. 57

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ANALYSIS OF SECTIONS OF LUNG

GROUP "C"

No. 61. Pneumonitis and bronchiolitis. Alveolar congestion marked.

No. 62. No lung on slide.

No. 63. Pneumonitis and bronchiolitis. Marked arterial engorgement.

No. 64. Mainly a bronchiolitis. Slight involvement of the alveoli.

No. 65. Pneumonitis and bronchiolitis. Marked arterial congestion.

No. 66. Apparently little change from the normal pattern.

No. 67. Diffuse pneumonitis with patches of broncho-pneumonia. Degeneration of the alveoli many of which are almost obliterated. Congestion extensive and solid both in the lung tissue itself and about the bronchioles.

No. 68. Pneumonitis.

No. 69. Acute extensive pneumonitis with marked alveolar distension. Cellular infiltration extreme. Exudate pronounced. Hyalinization marked.

No. 70. Hemorrhagic interstitial pneumonitis and bronchiolitis.

No. 71. Slight pneumonitis and bronchiolitis.

No. 72. Lung almost normal although slight cellular infiltration is seen in some areas.

No. 73. Apparently little change from the normal pattern.

No. 74. Mainly interstitial pneumonitis.

No. 75. METASTASES AND LUNG ABSCESS. KERATINIZED "CELL NESTS" NUMEROUS ALONG WITH CLUSTERS OF MALIGNANT CELLS. BREAKDOWN OF LUNG TISSUE VERY PRONOUNCED. SOLIDARITY OF TISSUE IN TUMOR AREA.

No. 76. Acute pneumonitis with marked alveoli distension. Cellular infiltration extensive. Exudate pronounced.

No. 77. Lung appears quite emphysematous and well-infiltrated with polymorphonuclear leucocytes.

No. 78. Pneumonitis and bronchiolitis. Much hemorrhagic congestion.

GROUP "C" - Cont'd.

No. 79. Interstitial pneumonitis.

No. 80. Pneumonitis with marked edema about the alveoli.

No. 81. Pneumonitis with much fibrinous exudate. Alveoli distended.

No. 82. No lung on slide.

No. 83. Apparently little change from the normal pattern.

No. 84. Pneumonitis and bronchiolitis. Marked hemorrhagic congestion.

No. 85. Very mild cellular infiltration of tissue.

No. 86. Pneumonitis and bronchiolitis with many areas of emphysema. Much hemosiderin.

No. 87. Pneumonitis with fibrinous exudate. Marked cellular infiltration. Extensive hemorrhages. Some areas of emphysema.

No. 88. Pneumonitis with much cellular infiltration and exudate.

No. 89. Pneumonitis with bronchiolitis.

No. 90. Broncho-pneumonia with extreme solidarity of lung tissue. Cellular infiltration extensive. Obliteration of bronchioles and alveoli.

NOTE:

THE ABOVE TABULATION REPRESENTS THE GENERAL ANALYSIS OF GROUP "C" SERIES AND REVEALS UPON MICROSCOPIC EXAMINATION:

No. 75. ALONE IN THE SERIES SHOWED METASTATIC DEPOSITS IN THE LUNG.

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ANALYSIS OF SECTIONS OF LUNG

GROUP "D"

No. 91. Pneumonitis and bronchiolitis. Extensive cellular infiltration, edema, and emphysema.

No. 92. Slight pneumonitis. Some cellular infiltration. Hemosiderin extreme.

No. 93. Acute pneumonitis with marked alveolar distension. Cellular infiltration extreme. Exudate pronounced. Areas of hyalinization.

No. 94. Apparently little change from the normal pattern.

No. 95. Apparently little change from the normal pattern.

No. 96. METASTATIC DEPOSITS IN LUNG ARE PRESENT BUT LIMITED IN LOCATION TO A SMALL AREA OF THE TISSUE. MAINLY COMPOSED OF MALIGNANT CELLS. NO EVIDENCE OF ANY KERATINIZED "CELL NESTS."

No. 97. Broncho-pneumonia. Alveoli considerably broken down. Little intra-alveolar exudate. Interstitial cellular infiltration.

No. 98. Pneumonitis and bronchiolitis. Some emphysema.

No. 99. Pneumonitis and bronchiolitis.

No. 100. Broncho-pneumonia. Solidarity of alveoli. Marked cellular infiltration and extensive emphysema.

No. 101. Pneumonitis showing hyalinization of alveoli at apex. Cellular infiltration.

No. 102. Acute pneumonitis. Exudate excessive. Marked interstitial cellular infiltration.

No. 103. Broncho-pneumonia. Extensive cellular infiltration. Some emphysema.

No. 104. Pneumonitis and bronchiolitis.

No. 105. Pneumonitis and bronchiolitis. Some areas of emphysema.

No. 106. Acute pneumonitis with extreme cellular infiltration. Exudate very pronounced. Lung markedly congested.

No. 107. Slight pneumonitis and bronchiolitis.

No. 108. Broncho-pneumonia. Solidarity of lung at apex very pronounced. Intercellular edema. Some emphysema.

GROUP "D" - Cont'd.

No. 109. SMALL PATCH OF METASTASIS IN LUNG SUBSTANCE ABOUT A BRONCHIOLE. ALSO MASSES OF EXTENSIVE METASTASES COMPOSED MAINLY OF MALIGNANT CELLS. SOME EARLY KERATINIZATION. EVIDENCE OF BRONCHO-PNEUMONIA.

No. 110. SMALL PATCH OF METASTASIS COMPOSED MAINLY OF MALIGNANT CELLS. NO "CELL NESTS." THIS IS IN CENTRAL AREA OF TISSUE. ALSO AN AREA OF EXTENSIVE METASTASIS COMPOSED MOSTLY OF MALIGNANT CELLS, ALTHOUGH THERE IS SOME EARLY EVIDENCE OF KERATINIZATION IN THIS REGION.

No. 111. Marked hyalinization. Extreme interstitial exudate. Acidophilic staining marked.

No. 112. Pneumonitis. Cellular infiltration. Edema of alveoli.

No. 113. Pneumonitis and bronchiolitis.

No. 114. Pneumonitis and bronchiolitis.

No. 115. Interstitial broncho-pneumonia. Breakdown of alveoli. Little intra-alveolar exudate. Marked cellular infiltration. Many areas are solid with cells.

No. 116. MARKED METASTASIS WITH LARGE AREAS OF TISSUE DESTRUCTION. CELLULAR INFILTRATION EXTREME. EXUDATE MARKED.

No. 117. Slight pneumonitis.

No. 118. Slight cellular infiltration about vessels and bronchi.

No. 119. SMALL PATCH OF METASTASIS AROUND A BRONCHIOLE. SOME CELLULAR INFILTRATION IN OTHER AREAS.

No. 120. Broncho-pneumonia. Fibrinous exudate. Much deposit of hemosiderin.

NOTE:

THE ABOVE TABULATION REPRESENTS THE GENERAL ANALYSIS OF GROUP "D" SERIES AND REVEALS UPON MICROSCOPIC EXAMINATION THAT:

No. 96
No. 109
No. 110 ALONE IN THE SERIES SHOWED METASTATIC DEPOSITS
No. 116 IN THE LUNG
No. 119

SUMMARY BY GROUPS

GROUP "A" in which thirty (30) mice (controls) received METHYLCHOLANTHRENE twice a week.

RESULTS

No. 16 showed extreme metastases comparable to the growth characteristics as seen in the primary skin tumor. A greater portion of the lung tissue was involved. Keratinized "cell nests" were abundant and the general malignant pattern was typical.

No. 23 shows characteristics not as extreme as in No. 16 but more localized. In this case the "cell nests" are less keratinized and the lung tissue is indented from its surface inwards and there are definite necrotic changes. Other malignant characteristics are well defined.

No. 25 shows two well circumscribed areas of malignancy with very pronounced keratinized "cell nests" formations. These keratinized areas are well defined in their acidophilic staining qualities. The cells throughout the tumor mass are pleomorphic, anaplastic and reveal much hyper-chromatism.

GROUP "B" in which thirty (30) mice inhaled tobacco smoke (in a closed smoking chamber) for a short period each day and also received METHYLCHOLANTHRENE twice a week.

RESULTS

No. 35 shows extreme metastases in the lung involving a large area of tissue. In this section the lung is solid with growth and the keratinized "cell nests" are particularly large and confluent from the margin to the central region of the tissue. Beyond this the keratinized areas are smaller and the malignant cells are closely packed together and reveal anaplastic, pleomorphic and hyperchromatic characteristics.

No. 39. shows a dense mass of neoplastic growth almost obliterating the lung tissue. In addition to the extreme number of keratinized "cell nests" many show a disintegrated central portion (necrosis). The squamous cell characteristics are very pronounced and the overall cellular features show this growth to be not only extensive but a rather rampant process. The cellular appearances are the same as described in the other neoplasias.

No. 44. shows a very small patch of malignancy close to the lung apex. The keratinized "cell nests" in comparison with other growth descriptions are very small; however, there is no doubt about the metastatic nature of the area.

SUMMARY BY GROUPS - Cont'd.

No. 46 shows a metastatic area along the lung border of small dimensions but definite. This area is composed for the most part of malignant cells with similar characteristics referred to in the other metastatic growths with the one exception that there are no keratinized "cell nests" present.

No. 48 shows extreme metastases with similar characteristics as referred to in Section No. 35. The pattern and arrangement of the cells are the same and for that reason repetition is unnecessary.

No. 52. shows by far the largest area of involvement in this group. Even on macroscopic examination of the tissue two large masses are seen which upon microscopic examination show the same pattern of keratinized "cell nest" formations and the same striking effort at cell de-differentiation and keratinization. The anaplastic, pleomorphic and hyperchromatic characteristics are prominent among the malignant cells.

No. 57 shows extreme metastases in two areas, one of which is intimately related to a main bronchus. The squamous cell characteristics are well defined and again keratinized "cell nests" formations are present throughout the tumor. The malignant cells are closely packed in some areas and there is marked solidarity of the lung tissue. The same additional features mentioned in other growths are prominent in this section.

GROUP "C" in which thirty (30) mice were administered topically tobacco residue extract (acetone) and METHYLCHOLANTHRENE twice a week in that order.

RESULTS

No. 75 shows a large circumscribed mass of tumor with numerous small keratinized "cell nests" about a bronchus. The characteristics of the malignant cellular elements exhibit anaplasia, pleomorphism and hyperchromatism as is seen in other tumors of the series. Solid masses of inflammatory cells are present resulting in consolidation in some areas. Emphymatous changes are quite pronounced and in many regions the lung tissue shows considerable breakdown. A broncho-pneumonic pattern is quite evident.

GROUP "D" in which thirty (30) mice received topically acetone and METHYL-CHOLANTHRENE twice a week.

RESULTS

No. 96 shows a mass of malignant cells localized on the margin of the lung tissue. Although this area is small, many of the cells show a tendency to neoplastic characteristics. Numerous polymorphonuclear leucocytes are contained in the field. The change is not too striking from that of an inflammatory picture. Most of the lung tissue appears relatively normal except for local areas of cellular infiltration.

SUMMARY BY GROUPS - Cont'd.

No. 109 shows a small patch of malignancy within the lung tissue about a bronchiole. The cells reveal definite malignant characteristics in shape, size and staining appearances.

No. 110 shows a large mass of growth that is quite circumscribed and related to the apex of the lung. The tumor is composed for the most part of malignant cells which have caused extreme bulging of the tissue. No keratinized areas are present although there are patches of acidophile hyaline. The cells are unquestionably neoplastic in nature.

No. 116 shows multiple metastatic growths in both portions of lung tissue. Some detached portions of growth are seen about a bronchus. There are many areas of keratinized "cell nests" and the general malignant characteristics of the cells is very striking - anaplasia, pleomorphism and marked hyperchromatism. Many of the cells appear as tumor giant cells.

No. 119 shows a small patch of metastatic growth about a bronchus. Except for this area all other portions of lung tissue appear relatively normal.

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DISCUSSION

In analyzing the groups together rather than individually it will be noticed that with few exceptions there was present in each mouse evidence of respiratory pathology. Not all animals used in the course of the research died by virtue of the appearance or size of the tumor. A considerable number were put to death by artificial means to culminate the work within the allotted period of time.

It was also very surprising to note that irrespective of the size of the primary tumors, the number of visible microscopic metastases in the lungs were relatively few and these varied both in size and appearances. Naturally one might ask the question as to what number of metastases might have developed had the animals, whose deaths were produced artifically, had been permitted to survive to a natural death, by virtue of the effects of the neoplasms on their general body metabolism?

It seems rather interesting that Group "B" should have shown the greatest evidence of neoplastic changes in the lungs, and that these changes were unquestionable and vivid in some instances. The seven animals showing metastatic deposits in the lung apparently reacted differently over the same period, which can probably be based upon the assumption of varying resistances rather than upon size or location of the primary growth.

Group "D", which showed five cases of metastases in the lungs was again interesting when making a comparison with Group "B"; however, No. 116 was of especial interest by virtue of the size and overall involvement of the lung tissue.

It would appear that even though the incidence of the primary growth does not vary from the control groups, the degree of metastases does vary considerably, with, as has been stated, a more profound metastasis in Group "B". Again, all show the inflammatory condition regardless of the group and it is assumed that had the animals in Group "D" continued to survive they could well have caught up with Group "B".

It is very difficult to say whether the cells in the secondary areas are transported through the tissues to the lungs, since in every instance the primary tumor could be shelled out from its attachment to the thoracic wall. When the lungs were carefully examined, no evidence of adhesions were noticed during the autopsy of any animal. The other question that comes to mind is that concerning methylcholanthrene itself. Does it exert its influence via the circulation? This is difficult to prove because in order to arrive at a satisfactory conclusion one would have to ignore any local application and study lung response alone. Are these metastases <u>true</u> metastases? Naturally the question can only be determined when a greater number of animals are used.

Throughout this research the word METASTASIS appears quite consistently. Its use does not signify that secondary manifestations result from the primary lesions any more than the primary lesions could be considered secondary from the lung neoplasms. One can be specific and say that in many of the animals examined squamous cell carcinomas and adeno-carcinomas were found but their source of origin is undetermined. Whatever characteristics the lung neoplasms bear to the skin neoplasms is difficult to evaluate irrespective of any similarity in the microscopic appearances. Another important point to consider is the absence in the research of a <u>normal</u> factor.

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